Identification of Novel Multidrug Resistance-Associated Protein-3 (MRP3) Inhibitors in Rat and Human Hepatocytes in Suspension

Tom De Vocht[a], Christophe Buyck[b], Eef Hoeben[c], Pieter Annaert[a]

[a]Drug Delivery and Disposition, KU Leuven, Belgium
[b]Discovery Sciences, Janssen R&D, Beerse, Belgium
[c]Global Clinical Pharmacology, Janssen R&D, Beerse, Belgium

INTRODUCTION

- A significant number of preclinical and clinical studies pointed out that Multidrug Resistance-associated Protein (MRP) mediated efflux transport plays an important role in the systemic and tissue exposure profiles of many drugs and their metabolites and of endogenous compounds, like bile acids[1]. A problem associated with the MRP subfamily is that the **exact role** of the various **isoforms** in drug disposition is relatively **hard to study**, at least partly due to **lack of potent and selective MRP inhibitors**[2].

PURPOSE

- The purpose of this study was to **identify selective Mrp3 inhibitors** in **rat hepatocytes** in **suspension**, using the **oil spin method**[3].

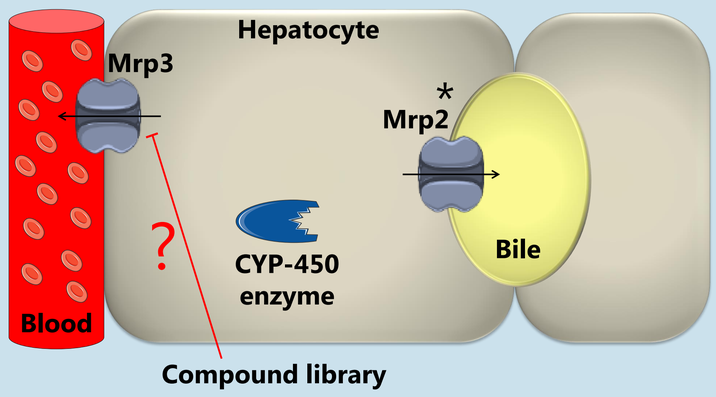
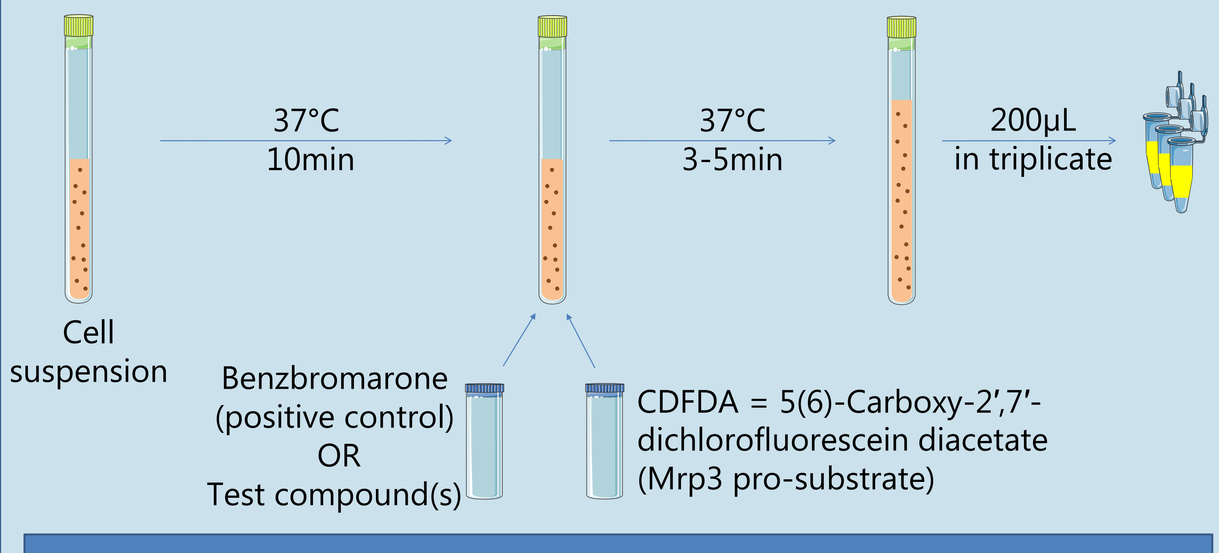


Figure 1: Graphical presentation of the purpose of this study. The aim is to identify selective inhibitors for Mrp3 using a screening approach in rat hepatocytes in suspension.
* In suspension, hepatic Mrp2 activity and expression is very low due to internalization implying that the present approach will elucidate mainly Mrp3 inhibitors[4].

METHODS

1) Pre-incubation step

2) Incubation step



3) Intracellular CDF quantification (Oil–spin method)

Incubation
CDFDA will react with intracellular esterases and CDF, benzbromarone or possible Mrp3 inhibitor with transporter

Centrifugation
To separate cells from suspension mixture

Quantification
Tube bottoms were cut, lysed and analysed by Fluorescence spectroscopy

Figure 2: Graphical presentation of the optimized assay used to screen for Mrp3 inhibitors in rat hepatocytes in suspension. The Spectrum Collection (MSDiscovery) and Janssen corporate compound collection were used as compound libraries. In total 1494 compounds were screened.

RESULTS

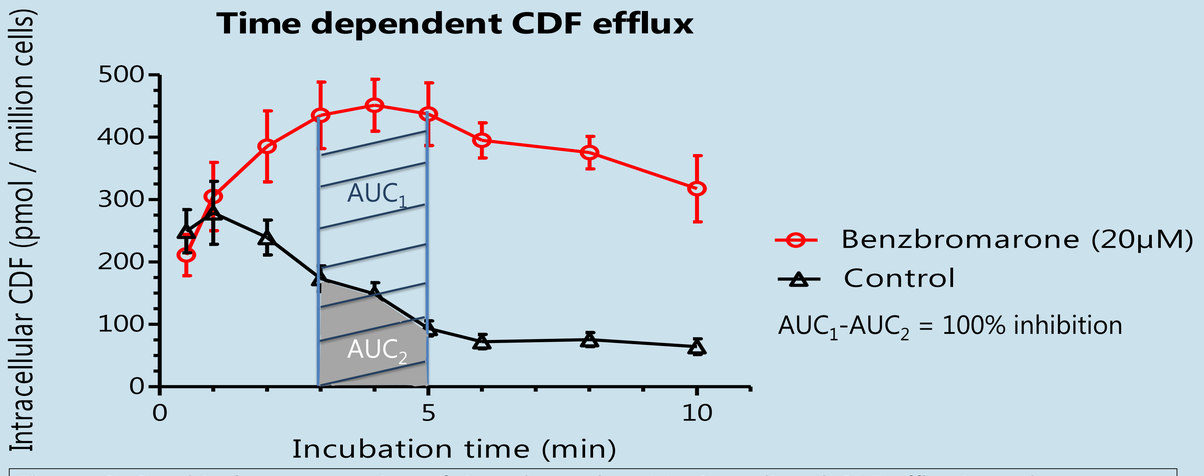


Figure 3: Graphical representation of time dependent Mrp3 mediated CDF efflux in rat hepatocytes in suspension. Based on the results, 3 min and 5 min were selected as time points for screening. The area under the curve (AUC) of benzbromarone subtracted by the AUC of the control condition represents 100% inhibition (n=3).

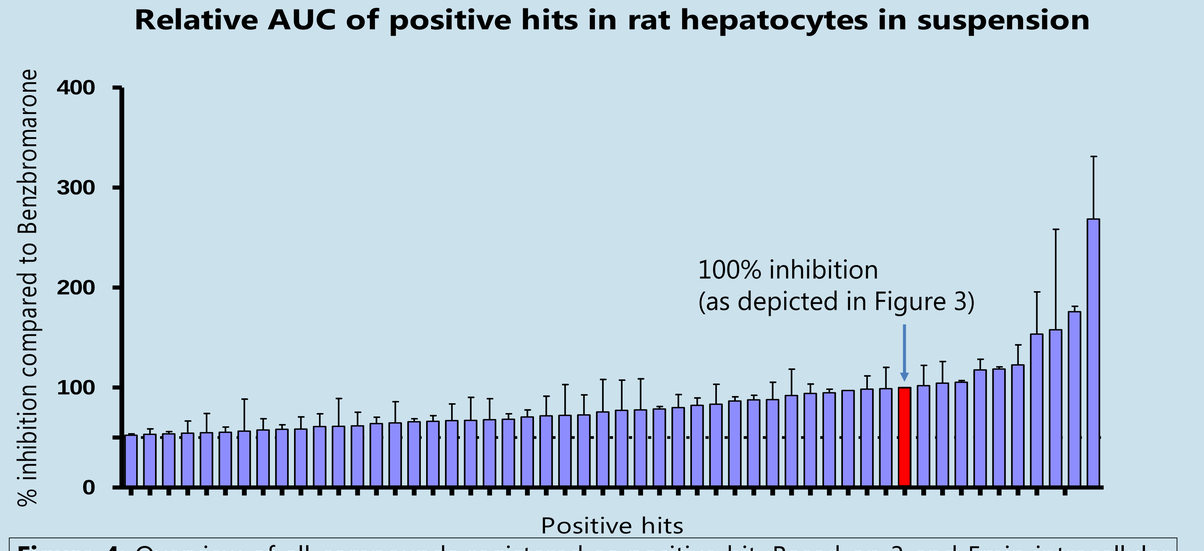


Figure 4: Overview of all compounds registered as positive hit. Based on 3 and 5min intracellular CDF levels, AUC values were calculated and expressed as % of the AUC obtained for benzbromarone (100%)(red bar). A 50% cut-off was chosen to distinguish between non-inhibitors and inhibitors. After screening 1444 compounds of The Spectrum Collection library and a hit rate of 2.5% (36 hits vs 1444 compounds screened) a Naïve Bayesian model in Pipeline Pilot was constructed. 50 compounds were selected out of Janssen library based on their chemical structures as being possible Mrp3 inhibitors based on this first model. This second screening resulted in a hit rate of 30% (15 hits vs 50 compounds screened) (n=2). In total, there was a hit rate of 3.4% (51 hits vs 1494 compounds screened). (n=2)

CONCLUSION

- Several strong Mrp3 inhibitors were identified using rat hepatocytes in suspension in this **optimized *in vivo* relevant *in vitro* assay.**
- **Human** hepatocytes are currently used with acetaminophen glucuronide as substrate which will allow to elucidate **cross-species differences** for Mrp3/MRP3 inhibition.
- **SAR modeling** will enable *in silico* screening of larger libraries and *in vitro* confirmation for more and potent Mrp3/MRP3 inhibitors.

REFERENCES

[1] Keppler D. Multidrug Resistance Proteins (MRPs, ABCCs): Importance for Pathophysiology and Drug Therapy. In: Fromm MF, Kim RB, editors. Drug Transporters [Internet]. Springer Berlin Heidelberg; 2011 [cited 2016 Mar 16]. p. 299–323. (Handbook of Experimental Pharmacology). Available from: http://link.springer.com/chapter/10.1007/978-3-642-14541-4_8

[2] Köck K, Brouwer KLR. A Perspective on Efflux Transport Proteins in the Liver. Clinical Pharmacology & Therapeutics. 2012 Nov 1;92(5):599–612.

[3] Fattah S, Augustijns P, Annaert P. Age-Dependent Activity of the Uptake Transporters Ntcp and Oatp1b2 in Male Rat Hepatocytes: From Birth Till Adulthood. Drug Metab Dispos. 2015 Jan 1;43(1):1–8.

[4] Bow DAJ, Perry JL, Miller DS, Pritchard JB, Brouwer KLR. Localization of P-gp (Abcb1) and Mrp2 (Abcc2) in Freshly Isolated Rat Hepatocytes. Drug Metab Dispos. 2008 Jan;36(1):198–202.

ACKNOWLEDGMENTS

- This work was supported by Janssen Research & Development and a research grant from the agency for Innovation by Science and Technology in Flanders, Belgium (N° IWT150724)

Contact: tom.devocht@kuleuven.be
Drug Delivery & Disposition
Herestraat 49, box 921
3000 Leuven, Belgium